II. NUCLEIC ACID TESTING OF BLOOD DONORS FOR HUMAN PARVOVIRUS B-19

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Nucleic Acid Testing for Human Parvovirus B19

Introduction

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Outline

- Introduction
- Scientific and Medical Review of B19

  Neal S. Young, M.D.
  Clinical Hematology Branch
  National Heart, Lung and Blood Institute, NIH
- Regulatory Framework and Question for the Committee

B-19 General Properties

- Non-enveloped
- 15-24 nm diameter; 5.6 x 10^6 daltons
- Two capsid proteins (VP1 and VP2)
- ssDNA (linear) ~ 5.6 kB
  - » plus/minus strands packaged independently
  - » self-priming terminal hairpins
  - » three overlapping open reading frames
- Heat stable
- Resists solvent/detergent

B-19 Infection: Normal Individual

- Early viremic phase
- Elaboration of IgM → IgG
- Clearance of virus
- Life-long immunity
- Symptoms
  - » Asymptomatic
  - » Erythema infectiosum (esp. children)
  - » Polyarthropathy (esp. women)
  - » Rheumatoid-like arthritis (resolving)
  - » Anemia (varying neutropenia and/or thrombocytopenia)

B-19 Infection among Blood Donors

- No screening of donors for B-19 in US (no licensed test)
- 40-60% of donors seropositive for anti-B-19 antibodies (most non-infectious; IGIV used to treat chronic B19 infections)
- 1:3,000-1:5,000 are viremic (PCR)
- 1:10,000-1:25,000 are high titer B-19 positive
- Viremia = up to 10^{14} genome-equivalents/ml
- Typically asymptomatic at time of donation

B-19 Transmission by Blood Products

- Transmission by blood components thought to be extremely rare
  - » Viremic Donor prior to seroconversion + Seronegative Recipient
  - » Exception: Pooled Plasma, Solvent-Detergent Treated, prior to the initiation of PCR testing for B19 DNA
- No confirmed reports of transmission by IG, IGIV, Albumin or PPF
- Significant transmission by clotting factor concentrates (VIII and IX)
  - » Prospective clinical trials
  - » Hemophiliacs seroconvert at a significantly earlier age than the general population

Minimizing Risk of B-19 Infection?

- Immunization
  - » Recombinant (VP1+VP2) vaccine in trials
  - » Ineffective in immune-compromised individuals
- Prophylactic IGIV
  - » Would require determination of individual's serology
  - » Would increase exposure to plasma derivatives
- Segregate B-19 negative lots and reserve for high-risk individuals
  - » Would require sensitive detection method for final product or segregation of high-titer pools
  - » Ethical and liability issues with distributing potentially infectious products

Minimizing Risk of B-19 Infection?

- Effective inactivation of B-19 during manufacture of factor concentrates
  - » Significant improvements in technology needed
- Reduce size of plasma pools from which factor concentrates are manufactured
  - » Only marginal reductions in risk would be achieved by extreme reductions in the scale of production
- Testing for B-19 antigen or DNA
  - » No screening test licensed in US
  - » NAT testing is more sensitive than antigen detection or DNA hybridization, but is not a routine assay method

Regulatory Issue Regarding NAT Testing for B19 DNA

Should FDA require studies under IND to validate B19 NAT testing?

- » Is it necessary to determine the clinical sensitivity, specificity and reproducibility of the test?
- » Are there clinical consequences of the performance or outcome of the test?
- » Are there informed consent issues?

Principal focus, at present, is on testing of plasma for further manufacturing (fractionation).

Human Parvovirus B-19 and the Safety of Plasma Derivatives

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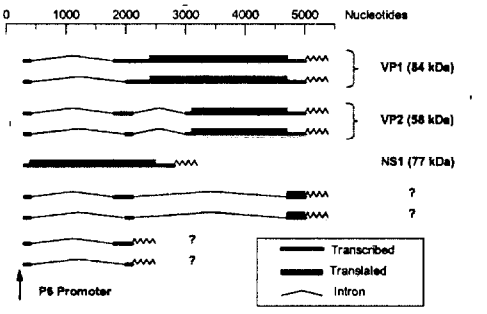
B-19 Genome Organization

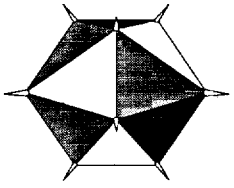


B-19 Proteins

Capsid Proteins

- VP1 84 kDa (781 aa)
  <4% of capsid mass
  Required for immunogenic capsids
- VP2 77 kDa (554 aa)
  >96% of capsid mass
  Lacks N-terminal 227 amino acids of VP1
  Insufficient for normal antigenic response

B-19 Structural Features



- Icosahedral capsid
- Projections at vertices
  - » Antigenicity
  - » Receptor recognition
- VP1:VP2::1:25
- VP2 alone is assembly competent
- VP1 + VP2 required to elicit immune response

B-19 Receptor

Blood Group P-antigen (globoside)
GalNac(β,1→3)Gal(α,1→4)Gal(β,1→4)Glc-ceramide

- No recognition of P_1 or P^k antigens (1:200,000 individuals are naturally resistant to B-19 infection)
- P-antigen expressed on:
  - » Erythroid progenitors, erythroblasts and erythrocytes
  - » Megakaryocytes
  - » Endothelial cells
  - » Placenta
  - » Fetal liver and heart

B-19 Transmission

- World-wide distribution
- Seasonal (late winter, spring)
- Four to five year cycles
- Transmitted by: Respiratory droplets
  Close contact
  Maternal→Fetal
  Transfusion

Cumulative B-19 Infections

Age	% Positive anti-B-19 Ig
< 5 yr	2-10%
15 yr	50%
> 20 yr	40-60%
Elderly	> 90%

1.5% annual seroconversion rate among women of childbearing age.

Consequences of B-19 Infection

Depends on Affected Individuals

- Normals
- Hemolytic anemia patients
- Pregnant women
- Immune compromised patients
  - » Immune suppressed
  - » Congenitally immune deficient
  - » AIDS

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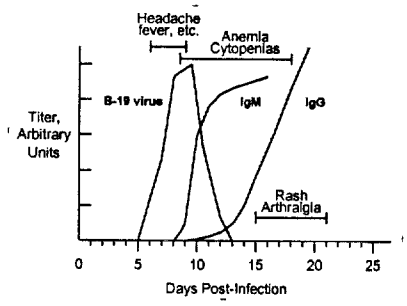

B-19 Infection Complicating Hemolytic Anemias

- Sickle Cell Anemia
- Hereditary Spherocytosis
- Thalassemia
- Red Cell Enzymopathy
- Autoimmune Hemolytic Anemia

Transient Aplastic Crisis (Pure Red Cell Aplasia)

B-19 Infection and Pregnancy

- Fetal Infection Rate ~ 33%
- Most severe fetal complications during 1st and 2nd trimesters
- Fetal Death (5-9%)
- Hydrops Fetalis (1:20,000-1:30,000 live births)
- Myocarditis
- Chronic post-natal infection

B-19 Infection of the Immune Compromised

- Typically no IgG, variable persistent IgM
- Cellular component of immune response likely but uncharacterized
- Chronic infection → chronic anemia (PRCA)
  - Severe, transfusion dependent
- Immunoglobulin therapy "always" effective
  - Reduction or clearance of virus
  - Relapse or reinfection possible

B-19 Infection among Blood Donors

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B-19 Transmission by Blood Products

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  - » Viremic Donor prior to seroconversion + Seronegative Recipient
  - » Exception: Pooled Plasma, Solvent-Detergent Treated, prior to the initiation of PCR testing for B19 DNA
- No confirmed reports of transmission by IG, IGIV, Albumin or PPF
- High frequency of transmission by clotting factor concentrates (VIII and IX)
  - » Prospective clinical trials
  - » Hemophiliacs seroconvert at a significantly earlier age than the general population

Prevalence of anti-B19 in Hemophiliacs Treated with Factor Concentrates (unheated)

STUDY	Hemophiliacs	Controls
Mortimer[1] (FVIII)	28/29 (97%)	18/92 (20%)
Bartolomei Corsi[2] (FVIII)	28/30 (93%)	17/58 (29%)
Williams[3] (FVIII)	40/45 (89%)	53/135 (39%)
Williams[3] (FIX)	7/8 (88%)	7/24 (29%)
Rollag[4] (FVIII)	118/192 (61%)	19/45 (42%)
Rollag[4] (FIX)	43/56 (77%)	-

B-19 (PCR) in Factor Concentrates

STUDY	Untreated	Heat-treated	Conditions
Zakrewska[1] (FVIII & FIX)	9/18 (50%)	0/7 (0%)	10 hr @ 60°C
McOmish[2] (FVIII)	11/18 (61%)	2/5 (40%)	72 hr @ 80°C*
McOmish[2] (FIX)	7/9 (78%)	-	
Lefrère[3] (FVIII & FIX)	6/28 (21%)	-	

* Approximately 2-log reduction in B-19 DNA after 72 hr @ 80°C; infectivity not determined.

Transmission of B-19 by Factor Concentrates

STUDY	Non-heated	Heat-treated
Bartolomei Corsi[1] (Steam/Dry)	28/30 (93%)	5/17* (29%)
Williams[2] (72 hr @ 80°C)	40/45 (89%)	2/12 (17%)
Lyon[3] (72 hr @ 80°C)	-	3/3 (100%)
Azzi[4] (10 hr @ 60°C)	5/7 (71%)	6/13 (46%)
Große-Bley[5] (Most 10 hr @ 60°C)	94/136 (69%)	20/32 (63%)
Santagostino[6] (30 min @ 100°C)	-	4/10** (40%)

* Seroconversion within 40 days of initiating treatment.
** Seroconversion within 2 weeks of initiating treatment.

Summary: B-19 Risk to Hemophiliacs

- Transmission by factor concentrates has been unequivocally demonstrated.
- No precise measure of risk associated with a single infusion:
  - Certainly greater than 1%; perhaps as high as 40%.
  - Long-term risk must approach 100%.
- Clearance during extensive purification does not provide an adequate safety margin.
  - » Nanofiltration is marginally effective due to small size of B19.
- Solvent/Detergent treatment is ineffective.

Summary: B-19 Risk to Hemophiliacs

- Data regarding the effectiveness of heat-inactivation are somewhat contradictory.
  - » Heat-treatment may reduce titers and rates of transmission but does not eliminate the risk.
  - » Impact on long-term risk is uncertain.
- Few reported cases have been symptomatic; most symptoms have been mild.
- Little is known of the consequences (if any) of repeat exposure to B-19 in factor concentrates.

Minimizing Risk of B-19 Infection?

- Immunization
  - » Recombinant (VP1+VP2) vaccine in trials
  - » Ineffective in immune-compromised individuals
- Prophylactic IGIV
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Nucleic Acid Testing (NAT) for Human Parvovirus B19

Regulatory Framework

Question for the Committee

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Regulatory Issue Regarding NAT Testing for B19 DNA

Should FDA require studies under IND to validate B19 NAT?

- » Is it necessary to determine the clinical sensitivity and specificity of the test?
- » Are there clinical consequences of the performance or outcome of the test?
- » Are there informed consent issues?

Principal focus, at present, is on testing of plasma for further manufacturing (fractionation).

Premises

- NAT for B19 DNA would reduce the viral load of the plasma pools from which plasma derivatives are made.
- Reducing the contamination of plasma pools by B19 would address a known risk of transmission associated with coagulation factor concentrates and pooled plasma.

Other Issues to be Considered in Adopting a Regulatory Strategy

- Testing Context
  - » Single donations vs. "minipools"
- Product Context
  - » Whole Blood Donations
  - » Recovered Plasma
  - » Source Plasma

  } Plasma for further manufacturing
  - » Final Products or Intermediates
- Precedent of NAT testing for HCV/HIV/HBV
  - » FDA determined that NAT for these enveloped viruses was to be considered "donor screening".
  - » Does the same rationale apply to NAT for B19?

Options for NAT of Plasma for Fractionation

- In-Process Control Test
  - » Requires validation as an analytical test (sensitivity, specificity and reproducibility)
  - » No clinical correlates need be established if :
    - No decisions regarding the management of the donor or recipient are based on the results of the test
    - No claims to enhanced safety are made
  - » Lower regulatory burden → faster implementation
- Donor Screening Test
  - » The severity of the infectious disease may warrant identifying and notifying the affected individual(s)
  - » Notification and followup may have a significant impact on the affected individuals, necessitating a determination of the clinical effectiveness of the test
  - » Clinical trials under IND with informed consent are needed to show clinical effectiveness

Elements of Assay Evaluation

Pre-Clinical	Clinical
• Specificity » Healthy Donors • Sensitivity » Known Positives • Analytical Specificity » Interference • Analytical Sensitivity » LLOD/LLOQ • Precision » Reproducibility and Proficiency	• Specificity » Healthy Donors » F/U testing • Sensitivity » High Risk Donors » F/U testing Numbers of units and donors to be included may depend on the virus and design of test (pooled vs. single unit).

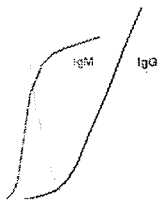
Rationale for Considering NAT Testing for HCV/HIV/HBV to be Donor Screening

- **Donor-related Criteria**
  - » Individual donor should be identified and notified
  - » Deferral, treatment, avoidance of 2° infections, donor's right to know
- **Recipient-related Criteria**
  - » Recipients of implicated products should be identified and notified
  - » Seek testing and, if positive, seek treatment and avoid 2° infections; recipient's right to know
- **Product-related Criteria**
  - » Quarantine/destroy the positive unit
  - » Quarantine/destroy other components from same (Whole Blood) donation
  - » Perform Lookback: retrieve/destroy unpooled or untransfused units

Relevant Aspects of HCV/HIV/HBV Infections

Characteristic	Consequences
Severe disease	Fatal or causing significant morbidity → need to notify
Long latency/window	Prior donations may be infectious despite negative test(s) → need for lookback
Chronic infections	Individual may remain infectious for life → need to defer donor permanently

B-19 Infection: Normal Individual



Relevant Aspects of B19 Infections

Characteristic	Relevance to B19
Severity of disease	Mild or asymptomatic in most cases
Latency/window	Short period before seroconversion; short duration of disease
Chronic infections	Rare in general population; Disease is self-limiting; Anti-B19 is beneficial for IG products

Criteria for Donor Screening - 1

Donor-related Criteria: Should individual donors be identified and notified?

Criterion	HCV/HIV/HBV	B19
Deferral	Yes	No[1]
Treatment	Where available	None usually indicated
Avoidance of 2° infections	Yes	No effective precautions*
Right to know	Yes	Yes**

* B19 is spread readily by casual contacts and an individual remains infectious for a short period of time.

** Although individual's interest in knowing may be less due to the less serious nature of the disease.

Criteria for Donor Screening - 1

Donor-related Criteria: Footnote 1

Clearly it is undesirable to defer permanently donors who test positive for a B19 infection. Normal individuals, following an acute infection, will mount an immune response that will neutralize the virus, limit the course of the disease and render the individual immune from further infections. The anti-B19 antibodies contributed by previously exposed donors are important for the efficacy of IGIV products for the treatment of B19 infections in sensitive individuals.

Because of the limits on the frequency with which Whole Blood donations may be made, it is highly unlikely that two consecutive donations of Whole Blood would both be infectious. Hence, there is no reason to defer, even temporarily, a Whole Blood donor based on a positive test for B19.

It is possible, however, that a Source Plasma donor may contribute so frequently that two consecutive donations could theoretically be infectious. In this case, there could be some benefit to deferring the donor temporarily (holding the donor in "abeyance").

Criteria for Donor Screening - 2

Recipient-related Criteria: Should recipients of implicated products[2] be identified and notified?

Criterion	HCV/HIV/HBV	B19
Testing	Yes	Less important in most cases
Treatment	When available	None usually indicated
Avoidance of 2° infections	Yes	No effective precautions*
Right to know	Yes	Yes**

* B19 is spread readily by casual contacts and an individual remains infectious for a short period of time.

** Although individual's interest in knowing may be less due to the less serious nature of the disease.

Criteria for Donor Screening - 2

Recipient-related Criteria: Footnote 2

Implicated products could include components made from the same (Whole Blood) donation, or components from previous donations identified through "lookback".

Because of the short duration of the window period of a B19 infection, lookback is not considered to be necessary.

Source plasma donations have no transfusable components associated with them, so recipient notification is not relevant.

With respect to components made from the same Whole Blood donation, some recipients of these components may be among the "at-risk" individuals (pregnant women, immune-compromised or -suppressed patients, patients suffering from hemolytic anemias). There may be some clinical benefit to these persons in knowing that they had received a B19-positive unit. The frequency with which such transfusions are made to non-immune, "at risk" individuals is unknown.

Criteria for Donor Screening - 3

Product-related Criteria: How should implicated units or products be dispositioned?

Criterion	HCV/HIV/HBV	B19
Quarantine/destroy positive unit	Yes	Yes
Quarantine/destroy units from same donation*	When possible**	When possible**
Lookback	Yes	No

* Relevant only for Whole Blood donations; no transfusable components are collected during Source Plasma donations.

** The ability to retrieve other components from a single Whole Blood donation depends on whether NAT testing can be completed before the expiration date of those components.

Conclusions - 1

- **On balance, public health considerations do not mandate the identification of a single B19-positive unit in a minipool, or the notification of the individual donor.**
- **Demonstration of clinical effectiveness of NAT for B19 DNA is not necessary for plasma for further manufacturing.**
- **The effectiveness of NAT for B19 DNA can be established by preclinical validation of the assay method.**
- **FDA can utilize the Biologic/Product Licensing mechanism to insure the effectiveness of NAT for B19 DNA.**

Conclusions - 2

- **Eliminating unnecessary regulatory burdens will expedite implementation of NAT for B19 DNA.**
- **Where NAT for B19 is positive and is completed on recovered plasma prior to the expiration of other components from the same donation, untransfused components should be retrieved and discarded.**
- **The more general question regarding NAT testing of Whole Blood donations is deferred for the time being.**

Question for the Committee

Does the Committee agree that, pending a policy on screening Whole Blood donations, FDA need not require studies to validate the clinical effectiveness of NAT for B19 DNA under IND for plasma for further manufacturing?

APPENDIX

Outline of Key Non-Clinical Elements to Licensing NAT for Viruses in Blood or Plasma Donations

Note

Although clinical trials are an important aspect to eventual licensing of NAT for HCV/HIV/HBV, they are not the only licensing requirement. Extensive information has to be developed and submitted to FDA for review in many areas including the quality and consistency of reagents, appropriate handling of samples, adequate recordkeeping and the performance of the assay itself. This same information would be required for NAT that was to be considered an in-process control (IPC). The following slides summarize the topics that need to be addressed in licensing NAT as an IPC, all of which have been discussed in greater detail in recent past BPAC meetings and two draft guidance documents.

Guidance Documents

- Draft Guidance for Industry in the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Nucleic Acid Sequences of Human Immunodeficiency Virus Type 1 (July 10, 1998).
- Draft Federal Register Notice: Nucleic Acid Testing of Plasma Pools (in preparation).

Manufacturing Issues

- Rationale/Design of NAT test
- Assay Optimization
- Sample Preparation
- Primers, probes, enzymes
- Reaction buffers and other components
- Controls, calibrators, quantification stds
- Capture probes, reporter molecules
- Specimen and kit stability
- Instrumentation, software validation

Performance Related Issues

- Assay validation (below)
- Specimen stability during collection, transport, processing and storage
- Quality control and stability testing of kit components or reagents
- GMP facilities (manufacturing and testing)
- Operator training and laboratory surveillance pre- and post-licensure
- Lot release testing using reference reagents and CBER panels

Pre-Clinical Evaluation

- Specificity: Testing of clinical specimens from random, healthy donors
- Sensitivity: Testing of known positives
- Analytical Specificity: Testing for interference
- Analytical Sensitivity: Testing of dilutional series of known positive specimens--LLOD/LLOQ
  - Seroconversion panels, low titer samples, dilutional panels, reference preparations
- Precision: Assay reproducibility and laboratory proficiency